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HATCHING AND NAUPLIAR DEVELOPMENT IN *CYCLOPS VERNALIS* (CRUSTACEA: COPEPODA) IN RELATION TO AVAILABLE DISSOLVED OXYGEN¹

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ABSTRACT. The eggs of *Cyclops vernalis* were gassed with varying quantities of oxygen and nitrogen, then allowed to hatch and the larvae to develop. A critical threshold of available dissolved oxygen was observed at 1.34 mg O₂/l; below this level, hatching dropped to 15%. At 0.38 mg/l hatching did not occur. A similar threshold of survivorship (23%) was observed at 1.34 mg O₂/l. Below this level, no organisms reached the first copepodid stage. Survival above 70% required 2.67 mg O₂/l dissolved oxygen. Most naupliar mortality occurred at the initiation of ecdysis reflecting the stress imposed by oxygen crisis. A detailed description of the gassing procedure and apparatus is provided.

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INTRODUCTION

Cyclops vernalis Fischer is generally considered a pond or small lake species of copepod zooplankton. In large lakes its distribution is usually confined to the near-shore or shallow basin water masses (Watson 1976, Heberger and Reynolds 1977). The ease of its collection and culture have resulted in its popularity for use in laboratory manipulation. Robertson et al. (1974) have provided a thorough and detailed report on culturing *C. vernalis* for laboratory experimentation. Their work placed special emphasis on the relationship

of temperature to adult longevity and clutch size, as well as duration and developmental rate of eggs carried by females. The report provided no data on the influence of environmental oxygen on the developmental process. Although the work reported in this paper was conducted before their report was published, our results compliment their data and hopefully will contribute to understanding the adaptation by this important zooplankter.

METHODS AND MATERIALS

EXPERIMENTAL ORGANISMS. Our experimental stocks of *Cyclops vernalis* were collected from a small pond in Montgomery County, Ohio. The copepods were fed 3 times weekly. Pure cultures of *Euglena gracilis* provided food for the larval copepods while

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newly hatched *Artemia salina* were fed to the advanced copepodid and adult stages (Lewis et al. 1971). The *Artemia* nauplii were centrifuged and washed before being fed to the copepods. We filtered tap water through sand, carbon, and paper filters, and aerated it for at least 24 hr before its use for culture purposes.

Prior to each experiment, ovigerous females were narcotized with carbon dioxide-saturated water. Newly produced egg sacs were carefully removed. Each egg sac was placed in a stoppered, 4-dr shell vial (fig. 1) containing 10 ml of degassed water. The vial was then gassed with the appropriate mixture of oxygen and nitrogen.

GASSING PROCEDURE. The details of our experimental technique are emphasized below for 2 reasons. First, it is important to understand that this was not a traditional closed-vial, before and after measurement of oxygen uptake. It was designed to provide a series of precise ambient oxygen concentrations in which copepod eggs and nauplii were allowed to develop. Second, a basic premise of this technique was that, because the eggs and nauplii represented such a small biomass per unit volume of the combined liquid and gas phases, the oxygen concentration remained stable between the initial gassing and the termination of the experiment. This was confirmed by actual measurement.

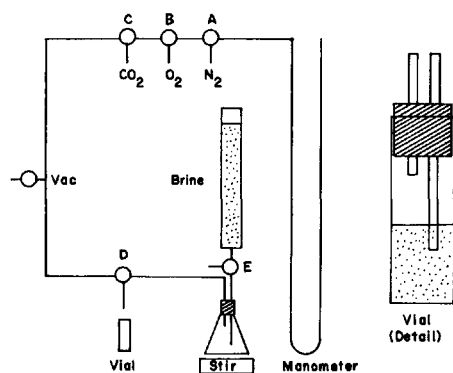


FIGURE 1. Schematic diagram of apparatus used to determine the influence of reduced oxygen on the development of *Cyclops vernalis* Fischer.

The procedure was as follows: The entire apparatus was flushed with nitrogen to assure that the system had no residual oxygen (fig. 1). The system was then evacuated down to approximately 636 mm of Hg. After evacuation, a calculated percentage of O₂ was added (valve B) to the system; then the correct amount of nitrogen was added (valve A) to restore the mixture to that day's atmospheric pressure level as read on the manometer. Carbon dioxide was not added to the experiments reported here. The system contained a 500-ml Erlenmeyer flask that

received the proper gas mixture. Here the gases were mixed thoroughly with a magnetic stirrer. Valve D was then turned to communicate between the flask and the outlet tube leading to the experimental vial. To expel the gas mixture, a saturated salt solution was added to the flask (valve E) thereby displacing and forcing the gas through the outlet tube. Two hundred ml of gas was bubbled slowly through the 10 ml of water in the experimental vial.

We used the microwinkler method (Burke 1962) to determine oxygen concentrations throughout the experiments. Repeated measurements confirmed that the relationship of the oxygen concentration in the gas phase to the dissolved oxygen in the culture water was linear throughout the entire range of experimental conditions.

EXPERIMENTAL CONDITIONS. Each vial was sealed off and placed on a 1/5 RPM rotator exposed to 10 hr of light and 14 hr of darkness. Two 25-watt incandescent bulbs supplied illumination. Experiments were conducted at room temperature and the daily readings ranged from 20.2 C to 23.6 C.

The copepod eggs usually hatched between 24-28 hr after being placed into the vials. Upon hatching of the eggs, the vial was removed and the egg sac containing the translucent first and second egg membranes was transferred with 1 ml of bottom debris and water. This was placed into a Sedgewick-Rafter counting cell on a compound microscope and systematically scanned to locate and count all hatched and unhatched eggs and any dead nauplii. After counting, the water from the counting cell was transferred back into the vial from which it came. At this point, .2 ml of pure *Euglena gracilis* culture was added as food for the nauplii. The vial was then regassed and returned to the rotator. Each vial was visually inspected daily for living copepods. When the larvae died or reached the first copepodid stage, the vial was removed from the rotator and treated with a few drops of 10% formalin. The contents of the vial were centrifuged and concentrated into one ml of water. The solution was then placed into the counting cell and the number of copepods counted and recorded.

RESULTS

The percent hatching of the eggs of *Cyclops vernalis* in relation to the amount of oxygen saturation in the water is shown in figure 2. There was a 48% increase in hatching between 1.15 and 1.34 mg/l oxygen (table 1). Above 1.34 mg/l oxygen, the ability to hatch increased as the concentration of oxygen increased until a plateau was reached at 2.48 mg/l oxygen. Figure 3 shows the percent survival to the first copepodid stage as a function of the availability of oxygen, and it is evident

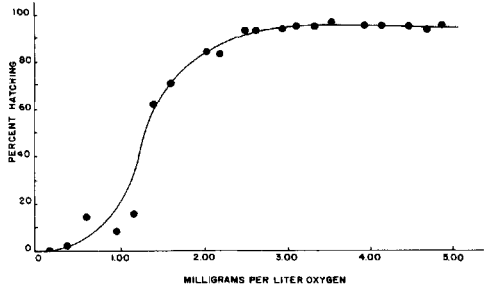


FIGURE 2. Percent hatching of eggs of the copepod *Cyclops vernalis* Fischer as a function of environmental dissolved oxygen. Each point of the graph equals approximately 135 individuals.

that below 1.34 mg/l oxygen no animals reached CI form. They may have completed a few molts, but did not develop to the copepodid form. Survival increased as the available oxygen increased to a plateau at about 2.67 mg/l oxygen with survivorship at approximately 80%. Below 1.34 mg/l oxygen, the copepods did not reach CI. Those animals that did, however, exhibited a constant rate of development (fig. 4). The period of larval life appears to be independent of the oxygen concentration in the surrounding media. Most of the animals tested completed their naupliar period in 12 days.

When the larvae died, their body contents burst out through the exoskeleton. This was a symptom of death for approximately 80% of the animals tested.

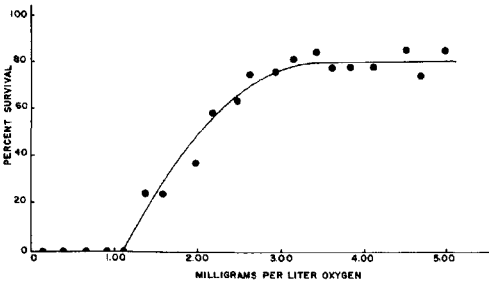


FIGURE 3. Percent survival of *Cyclops vernalis* Fischer to first copepodid stage as a function of environmental dissolved oxygen. Each point of the graph equals approximately 135 individuals.

TABLE 1
The influence of dissolved oxygen on the hatching, survival, and duration of larval life in Cyclops vernalis.

% Hatching	% Survival	Larval life (days)	Dissolved Oxygen (mg/l)
00	00	* 00.0	0.00
00	00	* 00.0	0.19
03	00	* 04.0	0.38
14	00	* 08.0	0.67
07	00	* 04.0	0.99
15	00	* 04.0	1.15
63	23	12.3	1.34
71	23	12.3	1.62
85	38	12.0	2.01
84	59	16.0	2.20
94	63	12.3	2.48
94	73	12.3	2.67
96	75	12.3	2.96
97	82	12.0	3.15
97	86	13.6	3.34
99	78	13.6	3.63
98	79	12.3	3.95
96	78	13.0	4.30
96	84	12.0	4.58
95	73	12.6	4.78
99	86	12.0	4.87

*These animals did not reach the first copepodid stage.

DISCUSSION

We have shown that for the eggs of *Cyclops vernalis*, hatching dropped to a very low percentage (15%) at oxygen concentrations lower than 1.34 mg/l, while at 0.38 mg/l, hatching did not occur. It is

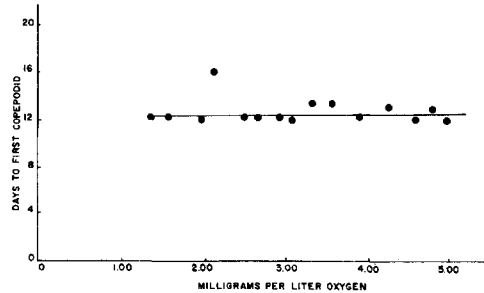


FIGURE 4. Duration of larval life of *Cyclops vernalis* Fischer as a function of environmental dissolved oxygen. Total mortality below 1.34 mg/l. Each point of the graph equals approximately 135 individuals.

apparent that the eggs were able to maintain normal development down to oxygen levels of about 1.34 mg/l. Below this level, respiration was possibly proportional to the environmental oxygen pressure and suggestive of the critical threshold of oxygen for the eggs of this species under the experimental conditions.

The percent survival to the first copepodid stage dropped abruptly at 2.01 mg O₂/l. At 1.34 mg O₂/l survivorship dropped quickly down to 23%. Below this point all copepods died before reaching first copepodid stage. Therefore, the critical threshold for the larval life of *C. vernalis* was about 2.0 mg O₂/l. Below this level, the copepod apparently cannot maintain aerobic processes and may begin converting their metabolism to an alternate pathway. Teal and Carey (1967) reported this behavior in *Uca pugilator*. In that crab, there is a decline of glycogen associated with an increase in lactic acid. Through anaerobic glycolysis, a copepod might be able to produce the ATP needed for survival for short periods of time. Marshall and Orr (1972) showed that *Calanus* has pathways available to convert ingested foods into lipids. It was observed that cyclopoids have fat droplets in the body of the larvae (where major development changes are taking place), as well as in the adult stage. Since this pathway exists in *Cyclops*, small amounts of oxygen could be obtained by this process. Teal and Carey (1967) found that the lipid content of *Uca* increased during anoxia as compared with animals in an aerobic situation. This may be the case with *Cyclops* and should be investigated further. This would be advantageous for *Cyclops*, and would allow it to acquire oxygen and some ATP through catabolism of carbohydrates during periods of low oxygen tensions.

During the premolt period of crustaceans, there is an increase in oxygen consumption (Tombes 1970). Both lipids and free amino acids are in small quantities during postmolt, increase during intermolt, and reach a peak in premolt. There

is a decrease of these materials as ecdysis approaches (McWhinnie et al. 1972). If during a period of low oxygen availability the general metabolism and growth processes are restrained, there could be a lower titer of these products (Skinner 1966). At other times in the copepods' life, a lower titer of these products may not be of great importance. Presumably, in premolt, glycogen is being deposited in the hypodermis, reusable nutrients are being reabsorbed from the old exoskeleton, and other metabolic processes are occurring. These factors in premolt can be very critical. Since amino acid utilization would be affected by the lower oxygen availability or by the lower metabolism rate associated with low oxygen, the splitting enzyme needed to weaken the cuticle may not be present or may exist in too little a quantity to facilitate ecdysis.

The duration of larval life for *C. vernalis* is apparently controlled by factors other than environmental oxygen. No larvae survived to the first copepodid stage below 1.34 mg O₂/l oxygen. The larvae that did survive to the first copepodid stage required about 12.7 days to reach this stage (fig. 4). Above this threshold, apparently internal mechanisms control the molting and development of the copepod.

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